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PCT/EP 2005/063457 70444 P2

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South Wales
NP10 8QQ

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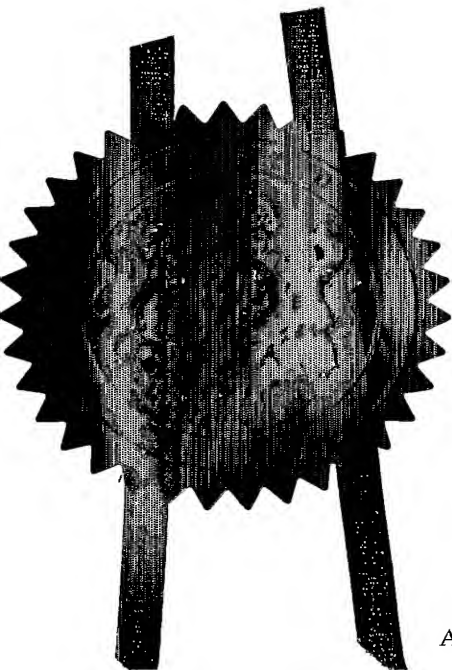
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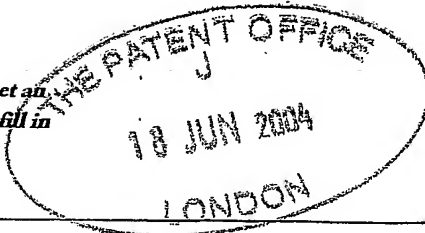
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0413698.2

3. Full name, address and postcode of the or of each applicant (underline all surnames)

SYNGENTA PARTICIPATIONS AG
Intellectual Property Department
Schwarzwaldallee 215
4058 Basel, SWITZERLAND

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

8029552001

4. Title of the invention

ORGANIC COMPOUNDS

Patent Office

5. Name of your agent (if you have one)

STEVEN PAUL WARD

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Syngenta Limited
Intellectual Property Department
Jealott's Hill Research Centre
PO Box 3538, BRACKNELL
Berkshire, RG42 6YA, UNITED KINGDOM

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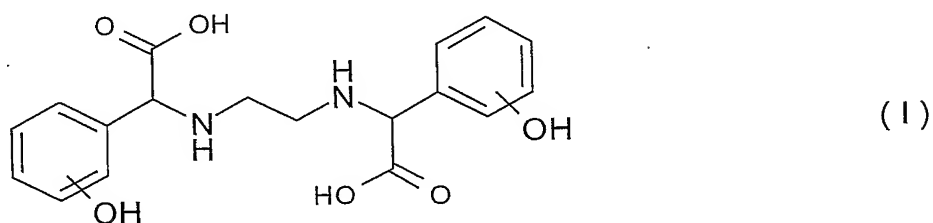
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Organic Compounds

The present invention relates to novel mixtures of N,N'-ethylene-bis(hydroxyphenyl)glycines, their metal complexes, their use in the field of agriculture as plant nutrient or component of fertilizers for plants and for treatment of chlorosis in plants.

N,N'-ethylene-bis(hydroxyphenyl)glycines (EDDHA) of formula I are known.

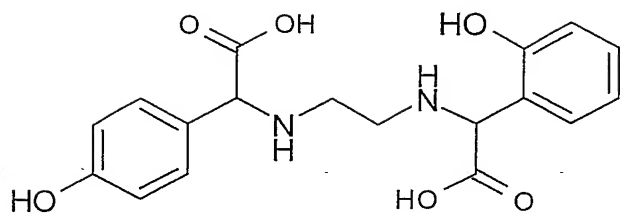


The position of the aromatic hydroxy groups at the various aromatic carbon atoms of the two separate phenyl rings allows the formation of different isomers. The predominant positions of hydroxy-substituents are the ortho- and para-positions at their respective phenyl rings.

One of the isomers is N,N'-ethylene-bis(4-hydroxyphenyl)glycine, in which both phenyl rings are para substituted with hydroxy (p,p-EDDHA). This compound is known in the art and is generally considered as unsuitable for complexing metals for the use in agriculture. A further isomer is N,N'-ethylene-bis(2-hydroxyphenyl)glycine, in which both phenyl rings are ortho substituted with hydroxy (o,o-EDDHA). This compound is well-known in the art and is often described as the most favourable compound for complexing metals, and being most suitable for the use as a plant nutrient or fertilizer because the complexation affinity to iron is particularly strong.

The ferric chelate of o,o-EDDHA shows a coordination number of 6 and is widely used for agricultural purposes as a plant nutrient or plant fertilizer, and for the treatment of chlorosis in crop plants. An illustrative picture of the ferric chelate of o,o-EDDHA is displayed, for example, in US-A-2921847.

Another isomer is N,N'-ethylene-(2-hydroxyphenyl)-glycyl-(4-hydroxyphenyl)glycine of formula II, in which one of the phenyl rings is ortho substituted with hydroxy while the other is para substituted with hydroxy (o,p-EDDHA).



(II)

Because the ferric chelate of o,p-EDDHA shows a coordination number of 5 and its complexation affinity to iron is weaker than that of o,o-EDDHA, this compound has often been seen as an undesired by-product being incurred by the manufacturing process of o,o-EDDHA.

However, minor amounts of o,p-EDDHA impurity are generally accepted and are in fact present in most commercial EDDHA products.

For reason of providing the pure product and achieving product homogeneity, most contemplated commercial processes are designed to avoid the o,p-EDDHA as by-product, for example the process disclosed in CH-A-633257.

For the treatment of plant chlorosis, a suitable chelating agent for iron should be

- a) sufficiently stable in the affinity to iron in order to avoid competition in complexation from other cations,
- b) it should be rather in-reactive with common soil materials,
- c) the affinity to Fe (II) should be low,
- d) it should not prevent iron reduction in dicotyledonous plants and
- e) should not compete with the natural chelating agents, phytosiderophores, in monocotyledonous plants (competition with phytosiderophores is one of the shortcomings of o,o-EDDHA) Additionally,
- f) the chelating agent should be able to release iron from the soil solid phase quite quickly.

The properties of the commercially available chelating agents on basis of EDDHA for the treatment of chlorosis of plants are in this aspect not completely satisfactory. Likewise, the availability of iron from such products to the treated plants is to some extent delayed, even where the intended administration to the plant aims at a quick supply with plant nutrient iron.

It is an object of this invention to provide an improved chelating EDDHA agent showing a combination of quicker iron release than known from the commercial chelating agents, with an unchanged spectrum of advantageous properties of the chelating agent which are described in the prior art.

It has surprisingly been found that employing certain amounts of the formerly undesired by-product o,p-EDDHA in iron chelating EDDHA products in fact improves the plant nutrient and fertilizing properties of said products.

5 According to present invention it is proposed to improve the plant nutrient and chlorosis treating properties of metal chelating EDDHA agents by utilizing an effective amount of o,p-EDDHA in a mixture comprising the o,o- and o,p-isomers of N,N'-ethylene-bis(hydroxyphenyl)glycines, wherein the molar ratio of N,N'-ethylene-(2-hydroxyphenyl)-glycyl-(4-hydroxyphenyl)glycine (o,p-EDDHA) to N,N'-ethylene-bis(2-hydroxyphenyl)glycine
 10 (o,o-EDDHA) is higher than 0.8 : 1, particularly of between 0.9 : 1 and 100:1. Preferably, the ratio of o,p-EDDHA to o,o-EDDHA is of from 1 : 1 to 50 : 1, or of from 2:1 to 10:1, or of from 0,9 : 1 to 2 : 1.

The present invention also relates to metal complexes of isomeric mixtures comprising
 15 N,N'-ethylene-bis(hydroxyphenyl)glycines, wherein the molar ratio of N,N'-ethylene-(2-hydroxyphenyl)-glycyl-(4-hydroxyphenyl)glycine (o,p-EDDHA) to N,N'-ethylene-bis(2-hydroxyphenyl)glycine (o,o-EDDHA) is higher than 0.8 : 1, particularly is between 0.9 : 1 and 100:1. Preferably, the ratio of o,p-EDDHA to o,o-EDDHA is of from 1 : 1 to 50 : 1, or of from 2:1 to 10:1, or of from 0.9 : 1 to 2 : 1.

20 The metal complexes of the EDDHA-mixtures according to the invention may with advantage be used as plant nutrients or components of plant fertilizers in agriculture. Preferred mixtures for this purpose comprise the ferric chelate of o,p-EDDHA and o,o-EDDHA in the ratio according to the present description of the invention. The ferric chelates are particularly
 25 useful as nutrients or components of fertilizers for agriculturally cultivated plants and for the treatment of chlorosis in agricultural crops..

Preferably, such metal complexes are the ferric EDDHA chelates. Such ferric chelates are not limited to chelates solely comprising iron as a metal, but they may contain further metals as well, preferably an alkali- or alkaline earth metal, like sodium, potassium, calcium or
 30 magnesium, particularly sodium. Preferably, the ferric chelate is a chelate of the type Na Fe (EDDHA), K Fe (EDDHA), or a mixture thereof.

The iron content of the ferric chelate is in general from 0.5 to 10 percent by weight, preferably from 1 to 8 percent by weight, in particular from 1.5% to 7%, in particular from 2%
 35 to 6% by weight or of from 2% to 5.5%, especially from 2.4% to 5.5% percent by weight.

With an analytical method which has especially been developed and adapted for this purpose, namely the isocratic ion-pair high-performance liquid chromatography, in contrast to the former state of the analysis technology the content of o,p-EDDHA and o,o-EDDHA can now be accurately determined both in quantitative and qualitative parameters, allowing to correlate the beneficial effect of mixtures comprising o,p-EDDHA to the molar ratio of o,p-EDDHA to o,o-EDDHA contained.

The analytical method is in principle known and has been published in Journal of Chromatography A, 1996, 727, 253-264 and in Journal of Chromatography A, 1997, 789, 453-460. The method described therein for the purpose of present invention has especially been adapted for the determination of EDDHA isomers.

This adapted method describes a procedure for the chromatographic determination of the total amount of chelated iron in samples containing o,o-EDDHA and o,p-EDDHA as chelating agents and the amount of their ferrous chelates, and is set out in detail in the following Examples.

The ratio of o,p-EDDHA to o,o-EDDHA in commercial products available hitherto has been determined with the newly developed analytical method under standard laboratory conditions, and showed that all ratios of o,p-EDDHA to o,o-EDDHA in the known products were lower than 0.7 : 1.

The mixtures according to the invention are generally applied in form of diluted aqueous suspensions obtained from standard formulations and may be applied to the plants which are intended to be treated, or to the area where the plants are growing. When simultaneous administration of other agriculturally used active ingredients is intended the application can be done in any desired successive order, or even in form of a tank-mix prepared from commercial formulations. Alternatively, the EDDHA mixtures according to this invention may in selected cases be applied in solid formulations, i.e. as dusts or as granulates which may require to be mechanically incorporated into the soil.

The other agriculturally used active ingredients may be additional fertilizers, micronutrient donors or other preparations that influence plant growth and development. Likewise, it is also possible to apply simultaneously plant protection products like selective herbicides or insecticides, fungicides, bactericides, nematocides, molluscicides, or mixtures of several of such preparations, if desired.

The formulations of the isomeric mixtures of EDDHA according to present invention may further comprise agriculturally acceptable carriers, surfactants or other application-promoting adjuvants customarily employed in formulation technology.

Suitable carriers and surfactants may be solid or liquid and correspond to the substances ordinarily employed in formulation technology, such as e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, thickeners, binders or fertilisers. Such carriers and additives are described, for example, in WO 95/30651.

The EDDHA-mixtures according to the invention are used in unmodified form, i.e. as obtained from the production process in the technically pure form of the crude product or, preferably, together with the adjuvants conventionally employed in formulation technology, and are for that purpose advantageously formulated in known manner e.g. into emulsifiable concentrates, coatable pastes, directly sprayable or dilutable solutions, dilute emulsions, wettable powders, soluble powders, dusts, granules, and by encapsulation in e.g. polymer substances.

As with the nature of the compositions, the methods of application, such as spraying, atomising, dusting, scattering, coating, soil-drenching or pouring, are chosen in accordance with the intended objectives and the prevailing circumstances.

The formulations, i.e. the compositions or preparations comprising the mixtures according to the invention, may further comprise a solid or liquid adjuvant and are prepared in known manner, e.g. by homogeneously mixing and/or grinding the active ingredient with extenders, e.g. solvents, solid carriers and, where appropriate, surface-active compounds (surfactants).

Preferred further additive adjuvant components of compositions comprising the EDDHA-mixtures according to the invention are sodium lignosulfonate, dextrine, isooctylphenol polyglycol ether, sodium polycarboxylate 2,5-furandione polymer with 2,4,4-trimethylpentene and the sodium salt of dodecyl-benzenesulfonic acid

Further surfactants customarily used in formulation technology will be known to the person skilled in the art or can be found in the relevant technical literature.

Whereas commercial product preparations will preferably be formulated as concentrates, the end user will normally employ the chelating mixtures as dilute dispersions of the commercial formulations.

The formulations according to the invention normally comprise ferric chelates of the N,N'-ethylene-bis(hydroxyphenyl)glycines in a mixture ratio according to the invention of from 1 to

95 percent by weight, or of from 30 to 88 weight percent, or of from 35 to 73 weight percent, or of from 35 to 79 weight percent.

5 If the formulations are designed to comprise additional plant nutrients or plant fertilizers, these substances are preferably selected from the group including calcium sulfate CaSO_4 , calcium nitrate $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, calcium carbonate CaCO_3 , potassium nitrate KNO_3 , magnesium sulfate MgSO_4 , potassium hydrogen phosphate KH_2PO_4 , manganese sulfate MnSO_4 , copper sulfate CuSO_4 , zinc sulfate ZnSO_4 , nickel chloride NiCl_2 , cobalt sulfate CoSO_4 , potassium hydroxide KOH , sodium chloride NaCl , boric acid H_3BO_3 and metal salts
10 thereof, Na_2MoO_4 . The preferred additional nutrients may be present in an amount of 5% to 50% by weight, preferably of 10% to 25% by weight or of 15% to 20% by weight each.

Preferred additional nutrients are urea, melamine, potassium oxide, and inorganic nitrates. The most preferred additional plant nutrient is potassium oxide.

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Where the preferred additional nutrient is urea, which may be present in an amount of 1% to 20% by weight, preferably of 2% to 10% by weight or of 3% to 7% by weight..

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Preferred individual formulations according to the invention are compositions comprising

1 to 99 weight percent of ferric chelates of N,N'-ethylene-bis(hydroxyphenyl)glycines,
wherein the ratio of o,p-EDDHA to o,o-EDDHA is higher than 0.8:1;

0 to 20 weight percent of urea;

0 to 50 weight percent of potassium oxide; or

25

30 to 88 weight percent of ferric chelates of N,N'-ethylene-bis(hydroxyphenyl)glycines,
wherein the ratio of o,p-EDDHA to o,o-EDDHA is 1 : 1 to 50 : 1;

2 to 10 weight percent of urea;

10 to 25 weight percent of potassium oxide; or

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35 to 73 weight percent of ferric chelates of N,N'-ethylene-bis(hydroxyphenyl)glycines,
wherein the ratio of o,p-EDDHA to o,o-EDDHA is 2:1 to 10 : 1 or of from 0.9 : 1 to 2 : 1;

3 to 7 weight percent of urea;

15 to 20 weight percent of potassium oxide.

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The mixture according to the invention may contain an admixture of combinatory plant protective fungicides, resulting in some cases in unexpected synergistic activities. Such

mixtures are not limited to two active ingredients (one of formula I and one of the list of plant protective fungicides), but to the contrary many comprise more than one fungicide. Admixing components being particularly suited for this purpose include e.g.: Azoles, such as azaconazole, bitertanol, bromuconazole, cyproconazole, difenoconazole, diniconazole, epoxiconazole, fenbuconazole, fluquinconazole, flusilazole, flutriafol, hexaconazole, imazalil, S-imazalil, imibenconazole, ipconazole, metconazole, myclobutanil, oxpoconazole, pefura-
 5 zoate, penconazole, pyrifenoxy, prochloraz, propiconazole, prothioconazole, simeconazole, tebuconazole, tetraconazole, triadimefon, triadimenol, triflumizole and triticonazole; pyrimidinyl carbinols, such as ancymidol, fenarimol and nuarimol; 2-amino-pyrimidines,
 10 such as bupirimate, dimethirimol and ethirimol; morpholines, such as dodemorph, fenpropidine, fenpropimorph, spiroxamine and tridemorph; anilinopyrimidines, such as cyprodinil, mepanipyrim and pyrimethanil; pyrroles, such as fenpiclonil and fludioxonil; phenylamides, such as benalaxyl, furalaxyl, metalaxyl, mefenoxam (metalaxyl-M), ofurace and oxadixyl; benzimidazoles, such as benomyl, carbendazim, debacarb, fuberidazole and
 15 thiabendazole; dicarboximides, such as chlozolinate, dichlozoline, iprodione, myclozoline, procymidone and vinclozoline; carboxamides, such as carboxin, fenfuram, flutolanil, furametpyr, mepronil, oxycarboxin and thifluzamide; guanidines, such as guazatine, dodine and iminoctadine; strobilurines, such as azoxystrobin, dimoxystrobin (SSF-129), fluoxastrobin, kresoxim-methyl, metominostrobin, orysastrobin, picoxystrobin, pyraclostrobin
 20 and trifloxystrobin; dithiocarbamates, such as ferbam, mancozeb, maneb, metiram, propineb, thiram, zineb and ziram; N-halomethylthiotetrahydrophthalimides, such as captafol, captan, dichlofluanid, fluoromides, folpet and tolyfluanid; Copper-compounds, such as Bordeaux mixture, copper hydroxide, copper oxychloride, copper sulfate, cuprous oxide, mancopper and oxine-copper; nitrophenol-derivatives, such as dinocap and nitrothal-isopropyl; organo-
 25 P-derivatives, such as edifenphos, iprobenphos, isoprothiolane, phosdiphen, pyrazophos and tolclofos-methyl; various others, such as acibenzolar-S-methyl, anilazine, benthiavalicarb, blastidicid-S, boscalid, chinomethionate, chloroneb, chlorothalonil, IKF-916 (proposed name cyazofamid), cyflufenamid, cymoxanil, dichlone, diclomezine, dicloran, diethofencarb, dimethomorph, ethaboxam, fenoxanil, SYP-LI90 (proposed name: flumorph), dithianon,
 30 etridiazole, famoxadone, fenamidone, fentin, ferimzone, fluazinam, flusulfamide, fenhexamid, fosetyl-aluminium, hymexazol, iprovalicarb, kasugamycin, methasulfocarb, metrafenone, pencycuron, phthalide, picobenzamid, polyoxins, probenazole, propamocarb, pyroquilon, proquinazid, quinoxifen, quintozone, silthiofam, sulfur, triazoxide, triadinil, tricyclazole, triforine, validamycin, or zoxamide.

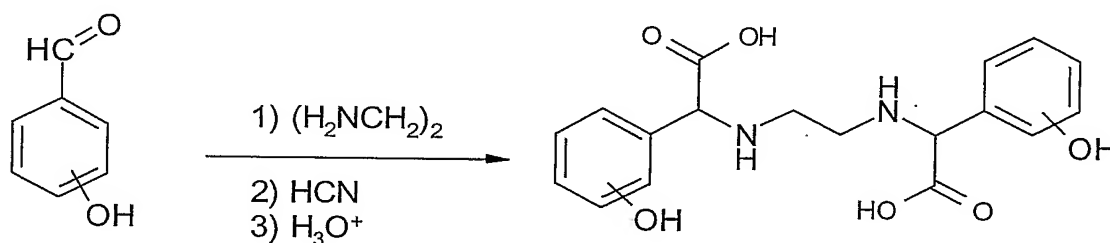
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In the above mentioned compositions with admixture of fungicide, the mixture ratio of the active ingredients is so selected that it reaches optional control of the phytopathogenic

microorganism on the host plants, which in turn allows the plant to fully exploit the benefit of receiving supplemental amounts of the metal from the EDDHA-chelate mixture. The mixture ratio is in general between 100:1 and 1:100, more preferably between 10:1 and 1:10 vis-à-vis the second fungicide. The mixtures may not only comprise one of the listed combinatory fungicidally active ingredients, but may comprise more than one additional active ingredients selected from that specified group, thus forming for example 3-way- or even 4-way-mixtures. The formulations according to the invention may further comprise herbicides, particularly sulfonyl ureas, as described in e.g. EP-A-837632.

For the production of the mixture according to the invention several processes are available. These processes are in general suitable to produce pure o,o-EDDHA and o,p-EDDHA. The pure products may be mixed afterwards to give the mixture with the desired ratio. Preferably processes are directly yielding a mixture according to the invention. For example, a process according to DE-1032748 may be employed, which is depicted in Figure 1.

Figure 1



In this process, two equivalents of o-hydroxybenzaldehyde or of p-hydroxybenzaldehyde, or preferably a mixture thereof are, in a first step, condensed with one equivalent of 1,2-diaminoethane and with elimination of water yields an aldimine. This reaction may be carried out under known standard reaction conditions. The water generated as one product during the reaction is preferably removed or trapped. Routinely this is done by standard laboratory methods like adding a drying agent, like magnesium sulfate or sodium sulfate, or by azeotropic distillation and removal of the water with a Dean-Stark trap. In the latter case dry benzene or toluene may be preferably used as solvents. In a second step, the product of the first step is reacted with hydrocyanic (prussic) acid. This step may be carried out by treating the aldimine product of the first reaction step with an aqueous solution of hydrocyanic acid in the presence of minor amounts of a mineral acid, like hydrochloric acid, at temperatures of about +20 to +25°C. Alternatively, an acidic aqueous solution of an alkali cyanide, like sodium cyanide, may be used as the cyanide source.

For the production of pure o,p-EDDHA only the details of the first reaction step have to be changed. In this case, the o-hydroxybenzaldehyde or the p-hydroxybenzaldehyde is employed in equimolar amount to the 1,2-diaminoethane, sufficient to condense one of the two amino groups, and after the reaction of these compounds is completed, the second equivalent of the respective other hydroxybenzaldehyde isomer is added and the reaction mixture is reacted further until completion.

It is also possible to react the hydroxybenzaldehyde with hydrocyanic acid yielding the corresponding cyanohydrins and to react the cyanohydrins with 1,2-ethylenediamine in a second step.

In a third step, the resulting ethylenediamine-N,N'-bis(hydroxyphenyl)acetonitrile may be hydrolysed by known methods, for example by reaction with concentrated hydrochloric acid at temperatures of +80 to +100°C. A base is then added to the reaction mixture until the pH value is 2 to 4, preferably 3 to 3.5. The precipitate is collected, then heated in concentrated hydrochloric acid yielding the dihydrochloride of the obtained N,N'-ethylene-bis(hydroxyphenyl)glycines. The free acid itself can finally be obtained by dissolving the dihydrochloride in excess sodium hydroxide solution and adjusting the pH value to 4.

Another process is described in *J. Agric. Food Chem.* **2002**, 50, 6395-6399. Pure o,o-EDDHA and pure o,p-EDDHA may be obtained with this process. The pure isomers may be mixed in the desired ratio to obtain a mixture according to the invention.

Another process to obtain o,o-EDDHA in a single-vessel reaction is described in US 4130582. This process for the preparation of EDDHA is designed to yield high purity o,o-EDDHA by suppressing the formation of p,p-EDDHA that would otherwise be predominantly formed. It comprises reacting an excess of phenol with ethylenediamine, glyoxylic acid in the presence of a base. The purity of the obtained o,o-EDDHA is above 95% when the content of water in the reactor is kept low, but deteriorates when a higher water content is tolerated. Surprisingly, it has now been found, that the mixture according to present invention may be synthesized in a single-vessel-reaction, suitable for up-scaling to a simple technical scale reactor size, which process comprises employing a 2- to 5-fold excess of the phenol starting material and balancing the contents of water in the reaction mixture in the range of 15% to 30 % by weight of the reaction mixture when reacting phenol with ethylenediamine and glyoxylic acid in the presence of a base.

Where particularly low amounts of phenol are utilized, no longer allowing to run the reaction in a melt of phenol or to properly handle the control equipment of the reactor, it may become

necessary to add an amount of inert organic solvent that allows adjust the reaction temperature to the range of +40°C to +80°C.

The mixture ratio of o,o-EDDHA and o,p-EDDHA content in the chelating agent according to this invention, may be adjusted according to the desired embodiment of present invention by applying the indicated variation of reaction conditions.

In particular, this process for the preparation of an EDDHA mixture according to present invention comprises reacting, at a temperature of from about +45°C to about +80°C., phenol with ethylenediamine, glyoxylic acid and a base. Phenol, ethylenediamine, glyoxylic acid and the base are preferably present in a molar ratio of 4 to 10 : 0.5 to 0.55 : 0.95 to 1.10 : 0.75 to 1.25. Preferred bases are alkali metal hydroxides, in particular sodium hydroxide.

A further suitable process for the preparation of the mixtures according to the invention is the conversion of o,o-EDDHA into o,p-EDDHA by changing the pH value at elevated temperature. The pH value for this conversion is of from pH 1 to pH 14, or of from over 7, in particular 8, to 14, or of from 9 to 13, at temperatures of from 50 to 120 °C, or of from 70 to 100 °C, or of from 80 to 90 °C for the time of about 5 to about 15, or 6 to 12, or 7 to 10 hours. In these conditions, in order to obtain a mixture according to the invention, the reaction has to be allowed to progress at the chosen conditions and then stopped when the desired mixture is obtained. In general, the reaction can be stopped by quenching the reaction mixture in water. Alternatively, the solution comprising o,o-EDDHA and o,p-EDDHA in the desired ratio may be used in a further process step to produce a metal complex, like a ferric chelate, by addition of iron(III)chloride. This process step can be carried out according to known processes.

Based on the the exact reaction conditions chosen, the skilled person establishes on the basis of various routine tests what reaction time is needed to obtain the desired ratio of o,o-EDDHA to o,p-EDDHA. The reaction mixture can be analysed by the procedure described in Journal of Agriculture & Food Chemistry, (2001) 49 (8) 3527-32).

By exposing o,o-EDDHA to these conditions it is converted to o,p-EDDHA and thus allows a convenient preparation of mixtures with ratios of o,o-EDDHA to o,p-EDDHA according to the invention. It is also possible to use a mixture of both EDDHA isomers and to raise the content of the o,p-isomer by this process. Metal salts of EDDHA, like iron salts, are also suitable as an educt for this process and may be used likewise.

Though the processes known from e.g. US 4,130,582 and references therein, are familiar to the artisan and require the same starting materials, there is no indication for the artisan that o,p-EDDHA could be obtained during the disclosed process.

- 5 Target plants in the agricultural practice, been in need of iron chelates as additional plant nutrient and agricultural crops suffering from plant chlorosis within the scope of this invention comprise, for example, the following species of plants: cereals (wheat, barley, rye, oats, rice, maize, sorghum and related species); beet (sugar beet and fodder beet); pomes, stone fruit and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries
10 and blackberries); leguminous plants (beans, lentils, peas, soybeans); oil plants (rape, mustard, poppy, olives, sunflowers, coconut, castor oil plants, cocoa beans, groundnuts); cucurbitaceae (marrows, cucumbers, melons); fibre plants (cotton, flax, hemp, jute); citrus fruit (oranges, lemons, grapefruit, mandarins); vegetables (spinach, lettuce, asparagus, cabbages, carrots, onions, tomatoes, potatoes, pepper); lauraceae (avocado, cinnamon,
15 camphor) and plants such as tobacco, nuts, coffee, sugar cane, tea, pepper, vinès, hops, bananas and natural rubber plants, and also ornamentals.

- A preferred method of applying the mixtures according to the invention, or an agrichemical composition comprising such a mixture, is application of an aqueous suspension to the soil
20 (soil applications such as a spray over the top or into the seed furrow) or as a soil drench, the frequency and the rate of application will be selected in view of the nature of the soil, the climate , the planted crop, the level of effect by chlorosis, and the maturity stage for the treated plant. The mixtures according to the invention may also be applied to foliage (foliar application), the frequency and the rate of application also depending upon the crop, the
25 level of chlorosis, the crop development stage for the situation question or to seed grains (coating) either by impregnating the grains with a liquid formulation of the active ingredient or by coating them with a solid formulation.

- Advantageous rates of application are generally between 1 kg and 50 kg of the ferric chelate of the isomer mixture of o,o- and o,p-EDDHA, per hectare (ha), preferably from 2.5 kg to 20
30 kg a.i./ha, especially from 5 kg to 10 kg a.i./ha.

When used as seed dressings, rates of from 0.001 kg to 1.0 kg of the isomer mixture of o,o- and o,p-EDDHA, particularly o,o- and o,p-EDDHA ferric chelates, per kg of seed are advantageously used.

- 35 The references cited herein are incorporated by reference for all useful purposes.

The following Examples only illustrate the invention described above, without limiting the scope thereof in any way. Temperatures are given in degrees Celsius.

Examples

5 Analytical method

The iron chelates are determined by linear gradient ion-pair high-performance liquid chromatography. When an iron chelate (anion) is added to a polar fluid (eluent), containing a large cation (ion-pair reagent), an ion pair is formed. This ion pair is retained by an apolar solid phase (stationary phase). The strength of the retention depends on the molecular size and its acidity. Then, each iron chelate presents a characteristic retention time and characteristic spectra depending on the chelating agent, and it is separated from the other substances present in the sample. The separation is carried out on a reverse phase silicagel column and an aqueous solution of TBA⁺ (tetrabutylammonium hydroxide) and acetonitrile as eluent.

The detection is based on photometry at 480 nm or a diode array detector. For Fe³⁺/EDDHA a gradient method or an isocratic one can be used. *Fe³⁺/EDDHA standard solution c(Fe) = 100 mg/l*

Instruction: Dissolve 0.1613g x 100 / R, where R is the complexometric purity of the standard in percentage of the acid form o,p-EDDHA obtained by photometric titration, with a Fe(III) standard in a pH stat system, of N,N'-ethylene-(2-hydroxyphenyl)-glycyl-(4-hydroxyphenyl)glycine (o,p-EDDHA) in 150 ml of water and 2.7 ml of NaOH (c=0.1 mol/l). After complete dissolution, 0.1271 g of iron chloride hexahydrate (FeCl₃·6H₂O) are added, stirring about 5 minutes. The solution is adjusted to pH 7,0 with NaOH solution (4.3). The solution is left overnight in darkness to allow excess Fe to precipitate as oxide and filtered quantitatively through a cellulose filter and made up to volume (250 ml) with water.

Eluent for the determination

Two solutions are used to make a linear gradient eluent:

Solution A: To 3.33 ml of TBAOH (40 wt.% Tetrabutylammonium hydroxide solution in water) are added 500 ml of water. The pH is adjusted to 6.0 with HCl. 350 ml of acetonitrile (HPLC grade) are added and made up to volume in a 1 l volumetric flask with water and filtered through 0.2 µm membrane filter.

Solution B: To 3.33 ml of TBAOH (40 wt.% Tetrabutylammonium hydroxide solution in water) 150 ml of water are added. The pH is adjusted to 6.0 with HCl and 750 ml of acetonitrile (HPLC grade) are added and the solution made up to volume in a 1 L volumetric flask with water and filtered through 0.2 µm membrane filter. TBACl or TBABr may also be used,

providing that pH is adjusted to 6.0 with NaOH. For Fe³⁺ /o,p-EDDHA determination, an isocratic method using only Solution A as eluent can also be employed

The Chromatograph is equipped with a gradient pump delivering the eluent at a flow rate of 1.5 ml/min. In table 1 the gradient table is presented.

Table 1 Gradient table

Time (min)	Flow rate (ml/min)	%A	%B	Curve type
0	1.5	100	0	Linear
5	1.5	100	0	Linear
6	1.5	0	100	Linear
11	1.5	0	100	Linear
12	1.5	100	0	Linear
20	1.5	100	0	Linear

For Fe³⁺ /o,p-EDDHA determination an isocratic elution can also be done using 100% A for 7 minutes. The chromatograph is equipped with an injection valve with a 20 µl injection loop. The column used is a C-18 column; 3,9 x 150 mm ID; dp = 5 µm (e.g.: SYMMETRY® C18, from WATERS) and a UV/VIS-detector with a 480 nm-filter or diode array, and an integrator.

Samples are prepared according top method 1 of Directive 77/535/EEC, OJ N° L 213,22.8.1977.

Preparation of the sample solution

125 mg within 0.1 mg are weighed into a 100 ml beaker (For samples with more of 4% of chelated iron, 80 mg and for samples with less 2% of chelating Fe 250 mg are employed). 80 ml of water are added and stirred for 30 minutes. After homogenization the solution is diluted to the mark with water.

Preparation of the calibration solutions

An amount of V1 ml (see table 2) of the chelate standard solution is pipetted into four 100 ml volumetric flasks and made up to volume with water and homogenized.

Table 2. Preparation of the calibration curve.

Solution	V1 (ml)	Concentration of Fe-EDDHA-standard	
		(mg Fe/ L)	(mmol/ L)
1	5	5.0	0.090
2	10	10.0	0.179
3	25	25.0	0.448
4	50	50.0	0.895

Chromatographic analysis

Immediately before injection, all solutions are filtered through a 0.45 µm membrane filter. The standard solutions (Table 2) are injected into the chromatographic system. Retention times are measured and the areas of the chelate (Fe³⁺ /o,p-EDDHA) chromatographic peaks are determined for all solutions.

A calibration graph is drawn with the values of the peak area of the standard solutions versus the corresponding chelating agent concentration (mg Fe/L or mmol/l).

Sample solution are injected and the chelating agent is identified by the retention time of the obtained peaks and if diode array detector is used, confirmed with UV-visible spectrum. Areas of the peaks are measured for the chelating agent. The concentration of the iron chelated (mg Fe/L) or the chelating agent (mmol/L) is determined using the calibration graph.

In case of the iron chelates the percentage by weight of the Fe (Fe(%)) as Fe³⁺ /o,p-EDDHA in the fertilizer is equal to:

$$\text{Fe(\%)} = C / m \times 0.01$$

Where:

C is the Fe concentrations (mg/L) of the Fe chelated by o,p-EDDHA determined with the calibration graph and *m*: is the mass of the sample taken for analysis in grams.

Preparation examples:Preparation of the N,N'-ethylene-bis[(hydroxyphenyl)acetonitrile]

A mixture of 109.8 g o-hydroxybenzaldehyde and 134.2 g p-hydroxybenzaldehyde in 600 ml of dry toluene is cooled to 0°C and a solution of 80 g ethylenediamine in 300 ml of dry toluene is added so that the temperature does not rise above +70°C. When the addition of the amine is completed, the mixture is refluxed with a Dean-Stark trap attached to the reflux condenser until no more water separated. The solvent and the excess amine are distilled off under reduced pressure. Yield: 90%.

To 268.1 parts by weight of the reaction product 700 parts by volume of liquid hydrocyanic acid, 180 parts by weight of water and 1.5 parts by volume of hydrochloric acid are added at a temperature of 0°C. The reaction mixture is stirred for three more hours. The reaction products forms during the reaction time and is filtered off. Melting point: 113-115°C.

Preparation of the N,N'-ethylene-bis(hydroxyphenyl)glycine

100 parts by weight of the N,N'-ethylene-bis[(hydroxyphenyl)acetonitrile] are heated in 1000 parts by volume of concentrated hydrochloric acid to +80 to +100°C for 3 hours. The educt dissolves slowly and after about ½ -1 hour another compound starts to precipitate. The reaction mixture is cooled and the precipitate is filtered off and washed with isopropanol. 100 parts by weight of the precipitate are taken up in water and heated to +100°C for 4 hours. Half of the water is distilled off and sodium hydroxide solution is added until the pH value reaches 3.5. A precipitate is being formed which is filtered off, re-dissolved in concentrated hydrochloric acid and heated to +100°C for 1 hour. The precipitate is dissolved in an aqueous sodium hydroxide solution and the pH value is adjusted to 4.0. The reaction product precipitates, is filtered and washed with isopropanol of a temperature of 0°C and dried. Melting point: 218-220°C, Yield: 31 %.

Preparation of the o,o- and o,p- mixture of N,N'-ethylene-bis(hydroxyphenyl)glycine

With the process described in US-A-4130582 and above, o,o-EDDHA has been prepared. With the same process o,p-EDDHA has been prepared by reacting equimolar amounts of o-hydroxybenzaldehyde and ethylenediamine. After completion of the reaction, an equimolar amount of p-hydroxybenzaldehyde was added and further reacted as described in US-A-4130582 to yield o,p-EDDHA. 10 g o,o-EDDHA and 10 g o,p-EDDHA were placed in a mortar and grinded thoroughly.

Preparation of the o,o- and o,p- mixture of N,N'-ethylene-bis(hydroxyphenyl)glycine in a single-vessel-reaction

A reaction vessel fitted with a condenser, dropping funnels and means for mechanical
 5 agitation was charged with 17 moles of molten phenol and retained at a temperature of
 +40-45°C. Then, 1 mole of ethylene diamine and, 50% aqueous sodium hydroxide solution
 containing 1.6 mole of sodium hydroxide and 10 moles of water (10mole) were added. After
 the reaction mixture was cooled to +40-45°C again, 50% aqueous glyoxylic acid containing 2
 10 mole of glyoxylic acid were added and the reaction mixture was heated to +70°C for 3 hours.
 The reaction mixture was then poured into excess water and the excess phenol was
 extracted with dichloromethane. The aqueous phase was examined by ¹H-NMR and found
 to contain a mixture of o,o-EDDHA and o,p-EDDHA in a ratio of about 1:1.

Preparation of the ferric chelate

15 To N,N'-ethylene-bis(hydroxyphenyl)glycine, sodium hydroxide solution is added until a clear
 solution is obtained. 40% technical iron(III)chloride solution is slowly added in an excess of
 27% while the pH value is kept at 7-8 by addition of further sodium hydroxide solution. The
 solution is evaporated to yield the ferric chelate of the type EDDHA Na Fe with an iron
 20 content of 5.3 %.

Conversion of o,o-EDDHA to o,p-EDDHA

2 g of o,o-EDDHA prepared according to US 4,130,582 were dissolved in water, yielding
 25 10ml of a 20% aqueous solution. The o,o-EDDHA solution was adjusted to pH 13 with 50%
 aqueous sodium hydroxide solution. The reaction mixture was heated to 80-90°C and stirred
 at this temperature for 7 hours. A sample of the reaction mixture was quenched in deuterium
 oxide and the pH adjusted to 0-1 with 98% sulphuric acid. The sample was analysed by
 proton-NMR according to Journal of Agriculture & Food Chemistry, (2001) 49 (8) 3527-32.
 30 The mixture was found to contain o,o-EDDHA and o,p-EDDHA in a ratio of 1:1.

Biological Examples

35 Soybean (cv.Oshumi) plants have been used in the biological experiment. Plants were
 obtained from soybean seeds that were germinated in standard seed growing procedure in
 closed sterilized trays. The seeds were placed in the trays on a cellulose paper soaked with
 50 milliliters of distilled water and with another paper placed over them. 30 ml distilled water

and 20 ml CaSO_4 1mM were added. The trays were placed in a thermostated stove, without light, at $+28^\circ\text{C}$ for 3 days. After this time the seedlings of similar development were placed on a holed plate floating over containers full of diluted nutrient solution and EDTA for 7 days. Nutrient solutions (without iron) were a mixture of: macronutrients: 1 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.9 mM KNO_3 , 0.3 mM MgSO_4 , 0.1 mM KH_2PO_4 ; cationic micronutrients: 2.5 μM MnSO_4 , 1 μM CuSO_4 , 10 μM ZnSO_4 , 1 μM NiCl_2 , 1 μM CoSO_4 , 115.5 μM EDTANa_2 (100 μM in excess to buffer micronutrients activities), 231 μM KOH ; anionic micronutrients: 35 μM NaCl , 10 μM H_3BO_3 , 0.05 μM Na_2MoO_4 . The pH was buffered at 7.5 with 0.1mM HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.05mM KOH and 2 g of CaCO_3 per pot.

Later, plants were subjected to a stressing procedure that consisted in putting them in five pails that contained 10 l each one of nutrient solution without iron chelate. The leaves were losing color indicating this way the iron chlorosis. After 6 days, the stems of two plants were wrapped together with foam and placed in 2l polyethylene vessels (3 holes per lid, 2 plants per pot). The pots were completely covered with black plastic to avoid the incidence of the light. The vessels contained 2 l of continuously aerated nutrient solution containing the Fe treatments. Nutrient solution consisted of macronutrients and anionic micronutrients as before and cationic micronutrients: MnSO_4 (1 μM), CuSO_4 (0.5 μM), ZnSO_4 (0.5 μM), NiCl_2 (0.1 μM) and CoSO_4 (0.1 μM). Water was added every two days and the solution was renewed every week. Plants were sampled 14 days after. Roots and leaves were separated and washed first with Tween® 80 (polyoxyethylene sorbitan monooleate) in 0.1M HCl for 30 seconds, and then with abundant distilled water, after that they were weighted and dried. Micronutrientes were determined in leaves and in roots after dry digestion procedure and atomic absorption. During the experiment SPAD readings were frequently taken using the Minolta SPAD 502 chlorophyll meter, for all the leaves.

Seven different treatments were applied:

Comparative Example 1: No iron added.

Comparative Example 2: Fe-o,o-EDDHA 5 μM

Comparative Example 3: Fe-o,o-EDDHA 5 μM + Fe-o,p-EDDHA 1 μM .

Comparative Example 4: Fe-o,o-EDDHA 5 μM + Fe-o,p-EDDHA 2.5 μM .

Comparative Example 5: Fe-o,o-EDDHA 10 μM

Example 1: Fe-o,p-EDDHA 5 μM .

Example 2: Fe-o,o-EDDHA 5 μM + Fe-o,p-EDDHA 5 μM .

Four replications were prepared for each treatment.

Table 3. Weight of fresh plant, fresh and dry leaf and fresh and dry root. (The number is the mean of 4 replicates)

Treatments	Fresh plant weight (g)	Fresh root weight (g)	Fresh leaf weight (g)	Dry leaf weight (g)	Dry root weight (g)
Comp. Example 1	5.133	2.859	1.116	0.339	0.264
Comp. Example 2	6.781	3.818	1.257	0.321	0.282
Comp. Example 3	12.383	5.245	3.877	0.602	0.364
Comp. Example 4	20.344	9.660	5.979	0.895	0.556
Comp. Example 5	15.862	7.316	4.736	0.781	0.425
Example 1	21.186	8.933	6.282	1.136	0.595
Example 2	22.001	10.089	6.499	1.092	0.643

Table 4. Iron content in parts of the plants. (The number is the mean of 4 replicates)

	$\mu\text{g Fe/ g dry root}$	$\mu\text{g Fe/ g dry leave}$	$\mu\text{g Fe/ number of leaves}$
Comparative Example 1	26.51	31.35	10.92
Comparative Example 2	90.32	62.30	19.82
Comparative Example 3	102.36	68.39	52.21
Comparative Example 4	98.46	74.12	64.06
Comparative Example 5	77.22	74.87	55.15
Example 1	128.48	80.98	91.91
Example 2	101.95	73.59	78.60

5 Table 5. Spad index obtained for the plants during the experiment (values for the second leaf). Means of 4 replicates.

Time / days	C1	C2	C3	C4	C5	E1	E2
0	20.8	12.7	16.8	18.1	16.6	14.7	17.2
2	19.2	10.1	15.5	17.6	15.7	14.0	16.9
4	17.2	9.1	16.2	19.8	15.0	18.0	23.8
7	14.4	9.5	18.0	22.0	18.2	21.4	27.7
9	13.6	9.2	18.1	24.1	19.7	23.9	30.6
11	12.8	8.2	18.2	22.7	19.0	24.9	30.5

In the biological examples it has been shown that treatment of the plants with ferric chelates of o,p-EDDHA results in a considerable raise of weight of the plants, see comparison of Example 1 with Comparative Examples 1 to 3 in Table 3. Mixtures comprising ferric chelates of o,p-EDDHA and o,o-EDDHA in a ratio of 1:1 also show an increased weight of the plants, see comparison of Example 2 with Comparative Examples 4 and 5 in Table 3. An increase in the iron content in parts of the plants under the conditions of the experiments can be seen in Table 4, comparing the values of Example 1 with Comparative Examples 1 to 3 and Example 2 with Comparative Examples 4 and 5.

Also the SPAD values, which are a measure for the chlorophyll contents of the leaves, raise considerably steeper in Example 1 in comparison to Comparative Examples 1 to 3 or Example 2 in comparison to Comparative Examples 4 and 5, respectively. The SPAD values after 11 days are also higher in the Examples than in the Comparative Examples. These results show a faster iron uptake of the plants in the presence of ferric chelates of o,p-EDDHA.

Patent claims

1. A mixture comprising isomeric N,N'-ethylene-bis(hydroxyphenyl)glycines, wherein the molar ratio of N,N'-ethylene-(2-hydroxyphenyl)-glyciny-(4-hydroxyphenyl)glycine (o,p-EDDHA) to N,N'-ethylene-bis(2-hydroxyphenyl)glycine (o,o-EDDHA) is higher than 0.8 : 1.
2. Metal complexes of mixtures comprising isomeric N,N'-ethylene-bis(hydroxyphenyl)-glycines, wherein the molar ratio of N,N'-ethylene-(2-hydroxyphenyl)-glyciny-(4-hydroxyphenyl)glycine (o,p-EDDHA) to N,N'-ethylene-bis(2-hydroxyphenyl)glycine (o,o-EDDHA) is higher than 0.8 : 1
3. Metal complexes according to claim 2, said metal complexes being ferric chelates of EDDHA.
4. Mixture according to claim 1, wherein the ratio of isomeric o,o- to o,p-EDDHA is of from 0.9 : 1 to 100:1.
5. Process for the preparation of a mixture according to claim 1 comprising reacting phenol simultaneously in a three component reaction with ethylenediamine and glyoxylic acid while selecting the reaction conditions in a way that directs the isomeric ratio of the generated EDDHA to be higher than 0.8 : 1.
6. Process for the preparation of a mixture according to claim 1 comprising the steps of reacting hydroxybenzaldehyde with diaminoethane in a first step, reacting the resulting aldimine with hydrocyanic acid and hydrolysing the resulting intermediate to yield the end-product.
7. Process for the preparation of a mixture according to claim 1 comprising conversion of o,o-EDDHA into o,p-EDDHA by changing the pH value at elevated temperature.
8. A formulation comprising the mixture according to claim 1 or a metal complex thereof, further comprising additional nutrients or plant fertilizers, herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides or mixtures thereof
9. A formulation according to claim 7 comprising urea, potassium oxide, an inorganic nitrate, a sulfonyl urea or a mixture thereof.

10. A formulation according to claim 7 or 8 comprising 1 to 99 weight percent of ferric chelates of N,N'-ethylene-bis(hydroxyphenyl)glycines, wherein the ratio of o,p-EDDHA to o,o-EDDHA is higher than 0.8:1; 1 to 20 weight percent of urea and 0 to 50 weight percent of potassium oxide
11. The use of a mixture according to any one of claims 1 to 9 as a plant nutrient or fertilizer, or for the treatment of chlorosis of plants.
12. The method of treating plant chlorosis in cultivated plants comprising administering to the plant or the area where it is planted an effective amount of a mixture according to any one of the claims 1 to 9 .



